DATA EVALUATION RECORD

1. **CHEMICAL:** Bromoxynil Octanoate
Shaughnessey Nos. 035301 and 035302

2. **TEST MATERIAL:** Bromoxynil Octanoate Technical; M & B Lot No. CN-51033 (20-DLM-152-1); 97.2% active ingredient; a brown solid.

3. **STUDY TYPE:** Growth and Reproduction of Aquatic Plants -- Tier 2. Species Tested: Navicula pelliculosa.

4. **CITATION:** Giddings, J.M. 1990. Bromoxynil Octanoate - Toxicity to the Freshwater Diatom Navicula pelliculosa. Report No. 90-8-3431. Conducted by Springborn Laboratories, Inc., Wareham, MA. Submitted by Rhone-Poulenc Ag Company, Research Triangle Park, NC. EPA MRID No. 416060-01.

5. **REVIEWED BY:**

Louis M. Rifici, M.S.
Associate Scientist II
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Signature: Louis M Rifici
Date: 2/11/91
Charles Lein 2/13/91

6. **APPROVED BY:**

Pim Kosalwat, Ph.D.
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Signature: P. Kosalwat
Date: 2/12/91

Henry T. Craven, M.S.
Supervisor, EEB/HED
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Signature: Henry T. Craven
Date: 2/19/91

7 **CONCLUSIONS:** This study is not scientifically sound. The test material had limited solubility and any precipitates present were not filtered away prior to measuring the concentration. The 120-hour EC_{50} of 0.051 mg a.i./L (measured) would be expected to have a detrimental effect on Navicula pelliculosa when applied at an application rate up to 0.375 lb a.i./acre (i.e. 0.275 mg a.i./L). The NOEC was determined to be 0.0093 mg a.i./L mean measured concentration.

Upgraded to core (see D170117)

8. **RECOMMENDATIONS:** N/A.

This

9. **BACKGROUND:**

10. **DISCUSSION OF INDIVIDUAL TESTS:** N/A.

11. **MATERIALS AND METHODS:**

A. **Test Species:** The alga used in the test, Navicula pelliculosa, came from laboratory stock cultures originally obtained from Carolina Biological Supply Company, Burlington, NC. Stock cultures were maintained in Algal Assay Procedure medium (AAP medium; USEPA, 1987) under test conditions. Transfers were made approximately once or twice a week. The culture used as inoculum was transferred to fresh medium twelve days before test initiation.

B. **Dosage:** Five-day growth and reproduction test. Based on the results of a preliminary test, six nominal concentrations of 0.016, 0.031, 0.063, 0.13, 0.25, and 0.50 mg a.i./L were selected for the definitive test.

C. **Test System:** Test vessels used were sterile 125-mL Erlenmeyer flasks fitted with stainless steel caps which permitted gas exchange. The test medium was the same as that used for culturing (excluding EDTA) with the pH adjusted to 7.5±1 with 0.1 N hydrochloric acid. Test vessels were maintained on an orbital shaker (shaking rate of 100 rpm) under continuous illumination (approximately 4-5 klux at the surface of the media) in a growth chamber.

D. **Test Design:** A 10 mg a.i./mL stock was prepared with 0.5145 g of Bromoxynil Octanoate Technical diluted to 50 mL in acetone. Appropriate volumes of primary stock were diluted to 10 mL with acetone to create secondary stocks. Equal volumes (0.05 mL) of the secondary stocks were diluted to 500 mL in sterile AAP medium. Solvent and media controls were also prepared. The solvent control contained 0.1 ml/L of acetone in algal medium which was equivalent to the concentration of solvent present in all test solutions. Three replicate 125-mL flasks (3 per treatment level and the controls) were conditioned by rinsing with the appropriate test solution. Fifty mL of the appropriate test solution were placed into each flask.

An inoculum of Navicula pelliculosa cells calculated to provide 0.3×10^4 cells/mL was aseptically introduced into each flask. The inoculum volume was 960 μL per flask. At each 24-hour interval, cell counts were

conducted on each replicate vessel using a hemacytometer and compound microscope. Upon test termination, the culture flasks were sonicated to separate the cells from the flask walls and break up clumps.

Water quality (pH and conductivity) was measured at test initiation and termination. Temperature was recorded continuously with a minimum/maximum thermometer. The shaking rate of the orbit shaker was recorded daily. The light intensity was measured at the beginning of the test and every 24-hour interval of the exposure period.

At test initiation, samples were removed from each test solution and the controls and frozen for subsequent analysis. Six quality control (QC) samples were prepared using fresh medium. The QC samples remained with the set of exposure solution samples throughout the analysis. At test termination, test solutions from each exposure level were composited and samples were taken from each composite for analysis. Three QC samples were also prepared (in fresh medium) at this time. All solutions were analyzed for Bromoxynil (as the phenolic degradate) by high-performance liquid chromatography (HPLC).

E. **Statistics:** For each observation period, the EC_{50} value and its 95% confidence limits were determined by linear regression of response (percent reduction of cell density as compared with controls) vs. mean measured exposure concentration over the range of test concentrations excluding controls. Various mathematical manipulations (logarithm and probit transformations) were used on the concentration and response data to get the linear regression with the highest coefficient of determination (r^2).

Cell density data were checked for normality and homogeneity of variance using the Chi-Squared Test and Hartley's Test (Neter et al., 1985), respectively. A t-test (Sokal and Rohlf, 1981) was used to compare controls with solvent controls. The no-observed-effects concentration (NOEC) was determined using one-way analysis of variance (Sokal and Rohlf, 1981) and Bonferroni's Test (Weber et al., 1989).

12. **REPORTED RESULTS:** The mean measured concentrations were 0.0093, 0.0018, 0.046, 0.086, 0.13, and 0.16 mg a.i./L. Measured concentrations at test initiation averaged 55% to

87% of nominal in the five highest concentrations. At test termination, measured concentrations averaged 55% of nominal except in the highest (0.50 mg a.i./L) and lowest (0.016 mg a.i./L) nominal concentrations. The highest nominal exposure solution was 11% of nominal at test termination, a value which was attributed to limited solubility of Bromoxynil under the test conditions. The lowest concentration, when measured at initiation and termination, was below the lower limit of detection established (lower than 84% of the nominal value of 0.016 mg a.i./L).

Cell densities determined at each observation time are presented in Table 3 (attached). Cell densities observed at 24, 48, 72, and 96 hours were very low (due to adherence of cells to the walls of the culture flask). Sonication greatly increased the number of cells counted at test termination. Cells appeared normal in all but the highest exposure level and cell density decreased as the Bromoxynil level increased.

The cell density data from the two sets of controls were pooled before determination of the NOEC. The data met the assumptions of normality and homogeneity of variance. The 120-hour EC_{50} was calculated to be 0.043 mg a.i./L (measured) with a 95% confidence interval of 0.013-0.14 mg a.i./L. The 120-hour NOEC was estimated as 0.0093 mg a.i./L (measured) using Bonferroni's Test.

Conductivity ranged from 170 to 190 µmhos/cm. The pH ranged from 7.4 to 7.5 in all test solutions and the controls at test initiation and between 7.5 and 8.0 at termination. The temperature ranged from 22 to 26°C during the study.

13. **STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:** No conclusions were made by the study author other than those included above.

Good laboratory practice and Quality Assurance Unit statements were included in the report indicating compliance to with EPA Good Laboratory Practice Standards under the Federal Insecticide, Fungicide, and Rodenticide Act (Federal Register, Part IV, 29 November 1983).

14. **REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:**

A. **Test Procedure:** The test procedure and the report were generally in accordance with the SEP and Subdivision J guidelines, except for the following deviations:

A twelve-day old culture was used as inoculum. A six

to eight-day old culture is recommended.

The light intensity during the test (4-5 klux) was higher than recommended (4.3 klux).

The dissolved oxygen, hardness, and alkalinity of the test solutions were not measured.

B. **Statistical Analysis:** The reviewer used a computer program (Toxstat Version 3.0) and the same methods cited in the report and obtained the same NOEC (see attached printouts 1 and 2). The reviewer used EPA's Toxanal computer program and determined the 120-hour EC_{50} as 0.051 mg a.i./L (95% C.I. = 0.039-0.067 mg a.i./L; see attached printout 3).

C. **Discussion/Results:** The solubility of Bromoxynil under the test conditions was cited by the report author as limited. The author suggests that limited solubility was the cause of lower recovery of the test material from the highest concentration (0.50 mg a.i./L, nominal) only. However, deviations from nominal concentration given in Table 2 (attached) were large for all test solutions and did not appear to follow any patterns. It is possible that precipitate was present in all or most test solutions.

Filtration of the HPLC samples, which could remove precipitates, was not mentioned in the report and, is therefore not assumed as being performed prior to analysis. When the test solutions were prepared for analysis, portions of the precipitated material may have been agitated into suspension and gone back into solution as a result of sample preparation. Therefore, the quantity of material measured would be more a function of random factors (e.g. how well the technician mixed the test solution before removing a sample) and the variability seen in the measured concentrations would be more easily explained.

The limited solubility of Bromoxynil under the test conditions and the lack of filtration of the samples prior to preparation for analysis suggests that the measured concentrations do not reflect the true concentrations in the test. Analysis of filtrates would have given a better indication of the actual quantity of test material present in solution.

The maximum application rate of Bromoxynil is 0.375 lb a.i./acre or 0.275 mg a.i./L if maximally applied to a

15-cm water column (as reported in another study, MRID #416060-05, with the same chemical and Anabaena flos-aquae). Under the conditions of this study, the 120-hour EC_{50} of 0.051 mg a.i./L is below the maximum application concentration. Bromoxynil Octanoate Technical would be expected to have a detrimental effect on this species and a Tier 3 evaluation is recommended.

D. Adequacy of the Study:

(1) Classification: ~~Invalid~~ Upgraded to core (See D170117)

(2) Rationale: Problem with the solubility of the test material. The actual exposure concentrations are not known.

(3) Repairability: No

15. COMPLETION OF ONE-LINER: Yes, 02/04/91.

Printout 1

BROMOXYNIL OCTANOATE, NAVICULA, MRID# 416060-01
File: a:41606001.nav Transform: NO TRANSFORM

t-test of Solvent and Blank Controls Ho:GRP1 MEAN = GRP2 MEAN

GRP1 (SOLVENT CRTL) MEAN =	41.4167	CALCULATED t VALUE =	-0.2554
GRP2 (BLANK CRTL) MEAN =	42.0000	DEGREES OF FREEDOM =	4
DIFFERENCE IN MEANS =	-0.5833		

TABLE t VALUE (0.05 (2), 4) = 2.776 NO significant difference at alpha=0.05

TABLE t VALUE (0.01 (2), 4) = 4.604 NO significant difference at alpha=0.01

Chi-square test for normality: actual and expected frequencies

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INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.407	5.082	8.022	5.082	1.407
OBSERVED	0	7	6	7	1

Calculated Chi-Square goodness of fit test statistic = 3.4821
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 20.43
Closest, conservative, Table H statistic = 184.0 (alpha = 0.01)

Used for Table H ==>	R (# groups) =	6,	df (# reps-1) =	3
Actual values ==>	R (# groups) =	6,	df (# avg reps-1) = (average df used)	2.50

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal but do not differ greatly, the Hartley test may still be used as an approximate test (average df are used).

Printout 2

BROMOXYNIL OCTANOATE, NAVICULA, MRID# 416060-01
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ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	2533.323	506.665	63.683
Within (Error)	15	119.344	7.956	
Total	20	2652.667		

Critical F value = 2.90 (0.05,5,15)
Since F > Critical F REJECT Ho:All groups equal

BROMOXYNIL OCTANOATE, NAVICULA, MRID# 416060-01
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BONFERRONI T-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	GRPS 1&2 POOLED	41.708	41.708		
2	0.0093mg a.i./L	38.417	38.417	1.650	
3	0.018	25.417	25.417	8.168	*
4	0.046	22.000	22.000	9.881	*
5	0.086	14.167	14.167	13.809	*
6	0.13	16.667	16.667	12.555	*

Bonferroni T table value = 2.60 (1 Tailed Value, P=0.05, df=15,5)

BONFERRONI T-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	GRPS 1&2 POOLED	6			
2	0.0093mg a.i./L	3	5.192	12.4	3.292
3	0.018	3	5.192	12.4	16.292
4	0.046	3	5.192	12.4	19.708
5	0.086	3	5.192	12.4	27.542
6	0.13	3	5.192	12.4	25.042

Printout 3

LOUIS M. RIFICI BROMOXYNIL OCTANOATE NAVICULA PELLICULOSA 2-1-19

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB.(PERCENT)
.16	100	100	100	0
.13	100	60	60.00001	0
.086	100	66	66	0
.046	100	47	47	0
.018	100	39	39	0
.0093	100	8	8	0

BECAUSE THE NUMBER OF ORGANISMS USED WAS SO LARGE, THE 95 PERCENT CONFIDENCE INTERVALS CALCULATED FROM THE BINOMIAL PROBABILITY ARE UNRELIABLE. USE THE INTERVALS CALCULATED BY THE OTHER TESTS.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 5.070616E-02

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
4	8.448331E-02	5.045353E-02	3.886825E-02	6.660919E-02

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
4	.6682925	13.22443	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 1.646387

95 PERCENT CONFIDENCE LIMITS = .3004795 AND 2.992295

LC50 = 4.371312E-02

95 PERCENT CONFIDENCE LIMITS = 7.727018E-03 AND .1349778

LC10 = 7.399898E-03

95 PERCENT CONFIDENCE LIMITS = 1.086984E-06 AND 2.143131E-02

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Page_____ is not included in this copy.

Pages 10 through 12 are not included.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

✓ FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Haughinessey No. 035301/035302 Chemical Name Bromoxynil Octanoate Chemical Class ____ Page 1 of 1

Study/Species/Lab/Accession	Chemical % a.i.	Results	Reviewer/Date	Validation Status
14-Day Single Dose Oral LD_{50}		LD50 = mg/kg (95% C.L.) Contr. Mort.(%)=		
Species	____	Slope= # Animals/Level= Age(Days)= Sex =		
Lab		14-Day Dose Level mg/kg/(% Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		
14-Day Single Dose Oral LD_{50}		LD50 = mg/kg. (95% C.L.) Contr. Mort.(%)=		
Species	____	Slope= # Animals/Level= Age(Days)= Sex =		
Lab		14-Day Dose Level mg/kg/(% Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		
8-Day Dietary LC_{50}		LC50 = ppm (95% C.L.) Contr. Mort.(%)=		
Species		Slope= # Animals/Level= Age(Days)= Sex =		
Lab	____	8-Day Dose Level ppm/(%Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		
8-Day Dietary LC_{50}		LC50 = ppm (95% C.L.) Contr. Mort.(%)=		
Species		Slope= # Animals/Level= Age(Days)= Sex =		
Lab	____	8-Day Dose Level ppm/(%Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		
~~48~~ 120-Hour LC_{50}		LC50 = 0.051 ppm (95% C.L. 0.039-0.067) Moving Average Inhibition Contr. ~~Mort.~~(%)= 0		
Species *Navicula pelliculosa*		cells/mL Sol. Contr. ~~Mort.~~(%)= 0		
Lab Springborn Laboratories	97.2%	Slope= N/A # Animals/Level= 0.3x10⁴ Inhibition Temperature = 22-26°C	LR 2/4/91	Invali
Acc. MRID 416060-01		48-Hour Dose Level ppm/(%~~Mortality~~ Inhibition) 0.0083 (5), 0.018 (39), 0.046 (47), 0.086 (66), 0.13 (60), 0.16 (100)		
		Comments: mean measured concentrations		
96-Hour LC_{50}		LC50 = pp__ (95% C.L.) Con. Mor(%)= Sol. Con. Mor.(%)=		
Species		Slope= # Animals/Level= Temp.=		
Lab	____	96-Hour Dose Level pp /(%Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		
96-Hour LC_{50}		LC50 = pp__ (95% C.L.) Con. Mort.(%)= Sol. Con. Mort.(%)=		
Species		Slope= # Animals/Level= Temp.=		
Lab	____	96-Hour Dose Level pp /(%Mortality) ( ), ( ), ( ), ( ), ( )		
Acc.		Comments:		